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Changes occurring in nutritional components (phytochemicals and free amino acid) of raw and sprouted seeds of white and black sesame (*Sesamum indicum* L.) and screening of their antioxidant activities

Tae Joung Ha, Myoung-Hee Lee[1], Woo Duck Seo[2], In-Youl Baek, Jae Eun Kang[3], and Jin Hwan Lee[3,*]

Division of R&D Performance Evaluation & Management, Research Policy Bureau, Rural Development Administration, Jeonju, Jeonbuk 54875, Korea
[1]*Upland Crop Breeding Research Division, Department of Southern Area Crop Science, National Institute of Crop Science, Rural Development Administration, Miryang, Gyeongnam 50426, Korea*
[2]*Division of Crop Foundation, National Institute of Crop Science, Rural Development Administration, Jeonju, Jeonbuk 54875, Korea*
[3]*Division of Research Development and Education, National Institute of Chemical Safety (NICS), Ministry of Environment, Daejeon 34111, Korea*

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*Corresponding Author
Tel: +82-42-605-7079
Fax: +82-42-605-7035
E-mail: schem72@korea.kr;
schem72@daum.net

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Abstract The present study is the first to investigate the germination properties regarding phytochemicals, amino acids, total phenolics, and antioxidant capacities of white and black sesame seeds. Nutritional components and antioxidant effects showed considerable differences. Sesamine and sesamolin composition decreased (white: 4.21→1.72, 3.57→1.57 mg/g; black: 2.43→0.58, 1.36→0.45 mg/g) during germination. Moreover, catechin displayed the predominant composition in sprouted seeds with values of 13.50 mg/g (white) and 19.09 (black) mg/g followed by (-)-epicatechin and sinapic acid. Total phenolics increased by approximately 4 times upon germination, i.e., 503.1±27.1→2085.0±56.7 (white) and 645.8±31.5→2480.1±49.5 (black), mg GAE/g. Amino acids also remarkably increased in sprouted white (7.04→31.69 mg/g) and black (6.55→26.97 mg/g) seeds, with individual composition occurring in the following order: asparagine>arginine>tryptophan>leucine>alanine. In particular, arginine and tryptophan exhibited the greatest variations. The antioxidant effects against DPPH radical were stronger in sprouted seeds depending on the phytochemicals. Therefore, sprouted sesame can be utilized as an excellent source for functional foods.

Keywords: germination, sesame seeds, phytochemical, amino acid, antioxidant activity

Introduction

In recent years, new safe and effective techniques in the food industry have been developed considering the increasing consumer demands for high nutritional quality (1). Food processing methods have also been developed to maintain high beneficial and nutraceutical values related to safety, non-toxicity, environmental friendliness, and possessing useful components for humans (2). For these reasons, many scientists have focused on food-processing skills to produce beneficial effects from vegetables, crops, and fruits (1-5). Among processing strategies, germination has been applied to several edible sources because it is an inexpensive and effective method for improving nutritional value and health-promoting functional properties in food (2,3,5). Moreover, this method has been known to produce various significant biochemical and physical reactions in crops and foods (5,6). It has been also demonstrated that germination can promote remarkable increases in vitamin, tocopherol, amino acid, and phytochemical contents (5,6). For example, germinated rice exhibits high GABA (γ-aminobutyric acid) content (7) and germination of legumes significantly increases phenolic compounds (2,3). Sprouted seeds are interestingly considered as a good source of several biological effects (2,3). Germinated *Brassica* vegetables play a vital role in the prevention of cancer (8) and sprouted millet has been reported to exhibit pharmacological properties including antioxidant and antimicrobial activities as well as prevent various chronic diseases (9). The extract of kidney bean showed high angiotensin-converting enzyme inhibitory and antioxidant capacities during germination (10). On the basis of the above reasons, germination process is an essential tool to improve the nutritional value and sensorial quality of food in the food industry. Among different nutritional components, phenolic compounds and amino acids have been considered as the principal factors to produce healthy foods. Phenolics are of great importance to the food industry because of their beneficial properties, including anticancer, antimutagenic, anti-

inflammatory, anticarcinogenic, and antioxidant capacities, positively affecting human health, (2,11-13). Amino acids are determinant metabolites of nutritional value for human health because of their basic composition of protein and energy source (14).

In our continuing survey on the development of dietary supplements and functional foods, the sprouted sesame seeds exhibited high nutritional qualities with respect to phenolic compounds and amino acids. Sesame (*Sesamum indicum* L.), belonging to the Pedaliaceae family, has been frequently used as a nutritious food in Asian countries (15). Nutraceutical characteristics of this crop, namely, antioxidant, anticancer, hepatoprotective, and hypocholesterolaemic activities, have demonstrated human health benefits (16,17). Furthermore, many researchers have reported that the positive biological effects of sesame seeds are linked to their metabolite contents such as phytosterols, lignans, and amino acids (18-20). Sesame leaves and flavors have been generally used as additives in foods (21) and sesame seeds are widely used in cosmetic and cooking preparations (21). Specifically, sesame seeds are known to possess many beneficial properties, including antimutagenic, hepatoprotective, and hypocholesterolaemic activities (16,17). The seeds are also an excellent food source for humans because of the presence of lignans and fatty acids, which play important roles in preventing heart disease and cancer (17,18-20). Although several researches have evaluated the nutritional components and biological effects of sesame seeds (16,17,20), there are few studies that have examined variations in the metabolites and beneficial properties of sprouted seeds during germination. Therefore, our study was designed to analyze the antioxidant capacities and nutritional compositions of raw and sprouted sesame with different seed-coat colors.

The current research was the first to investigate and compare the phenolic compounds and free amino acids in white and black sesame seeds during germination. Moreover, this study documented changes in the antioxidant properties against DPPH radicals and total phenolic content of raw and sprouted seeds.

Materials and Methods

Plant material and chemicals The two most frequently used sesame cultivars in Korea were selected for this study. White sesame (cv. Kopoom) and black sesame (cv. Miheuk) were developed by the National Institute of Crop Science, Rural Development Administration, Korea. The two cultivars were grown during 2012 in the experimental fields of this institute (Milyang city, Gyeongsangnam-do, Korea). The harvested sesame seeds were air-dried under natural light to remove the moisture and stored at −40°C. The 16 phenolic standards (gallic acid, *p*-hydroxybenzoic acid, catechin, gentisic acid, vanillic acid, caffeic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, salicylic acid, luteolin, cinnamic acid, and apigenin) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Sesamin and sesamolin standards were isolated by chromatographic techniques using silica gel column chromatography (230–400 mesh silica gel, kieselgel 60, Merck, Darmstadt, Germany) as described in a previous study (22). The 21 free amino acid contents (aspartic acid (Asp); threonine (Thr); serine (Ser); asparagine (Asn); glutamic acid (Glu); glycine (Gly), alanine (Ala); valine (Val); methionine (Met); isoleucine (Ile); leucine (Leu); tyrosine (Tyr); phenylalanine (Phe); γ-aminobutyric acid (GABA); ethanolamine (Ethamin); ammonium (Amm); lysine (Lys); 1-methylhistidine (1-Mhis); histidine (His); tryptophan (Trp); arginine (Arg)) were quantified using an amino acid standard of Sigma Chemical Co. Folin–Ciocalteu standard reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. Acetonitrile, acetic acid, lithium citrate buffer, and water were purchased from J.T. Baker (Phillipsburg, NJ, USA). Other chemicals, reagents, and solvents used in the present research were of analytical grade and were purchased from Sigma Chemical Co.

Instruments The isolated phenolic compounds, sesamin and sesaminol, were elucidated with a Bruker AM 500 (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz) spectrometer (Bruker, Karlsruhe, Germany) using dimethyl sulfoxide (DMSO)-d_6 with tetramethylsilane (TMS). Phenolic compound content was analyzed using a Dionex Ultimate 3000 High-performance liquid chromatography (HPLC) series (Dionex Softron GmbH, Germering, Germany), which included a dual low pressure ternary gradient pump and an Ultimate 3000 series photodiode array detector. Fatty acid composition was determined using gas chromatography (Agilent 7890A series; Agilent Technologies, Santa Clara, CA, USA). The concentration of free amino acids was determined by a Biochrom 30 series Amino acid analyzer (Biochrom Ltd., Cambourne, UK). Total phenolic content and antioxidant effect were measured using UV–vis absorption spectra on a Beckman DU650 spectrophotometer (Beckman Coulter, Brea, CA, USA).

Seed germination Sesame seeds were germinated according to the Pająk *et al*. method (22). Briefly, the seeds were sterilized for 2 min by immersion in ethanol and then steeped in deionized water at room temperature for 12 h. After steeping, the imbibed seeds were germinated in a growth chamber under controlled conditions (dark, temperature 25±1°C, humidity 80%) for 7 day. The sprouted seeds were immediately air-dried for 3 day at room temperature. The dried samples were weighted and finely ground and then stored in sealed bottles at –40°C for further analysis.

HPLC analysis for phenolic compounds The quantification of phenolic compounds was done using Ultimate 3000 HPLC analysis. A 20 μL sample of an 80% methanol extract was injected into an analytical Eclipse XDB-C_{18} column (150 mmx4.6 mm, 5 μm, Agilent Technologies). The mobile phase was composed of 0.1% acetic acid in water (eluent A) and 0.1% acetic acid in acetonitrile (eluent B). The gradient elution conditions were as follows: 0-3 min, 3% B; 7 min, 10% B; 20 min, 30% B; 22 min, 40% B; 25 min, 50% B; 30 min, 60% B;

40 min, 80% B, and then held for 5 min before returning to the initial conditions. The total running time was 40 min with a flow rate of 1.0 mL/min and the volume of injection was 20 μL. Moreover, the column temperature was maintained at 25°C and the detection of phenolics was monitored at 254 nm.

Preparation of sample and calibration curve for phenolic compound The dried sesame seeds were pulverized (60 mesh) using an HR 2860 coffee grinder (Philips, Drachten, Netherlands) for 5 min. The ground seeds (1.0 g) were extracted in 10 mL of 80% methanol in an ultrasonic bath for 6 h at 25°C. After centrifugation (at 3,000x*g* for 5 min) using VS-6000CFN (VISION, Seoul, Korea), the supernatant was filtered through a 0.45 μm syringe filter (Whatman Inc., Maidstone, UK). The filtrates were stored at 4°C until HPLC analysis. To analyze the phenolic compounds, the peak areas of phenolic standard materials were integrated with the HPLC chromatogram at 254 nm using Dionex software and plotted against the concentration to create a linear curve. Stock solutions of the standard material were prepared with DMSO to give a 1 mg/mL concentration and calibration curves were formed by diluting each stock solution in DMSO to six concentrations including 1, 5, 10, 20, 50, and 100 μg/mL, respectively. The correlation coefficients (r^2) of the 18 phenolic standards were detected to be higher than 0.998.

Determination of free amino acid Free amino acid was extracted from the lyophilized sample with 75% ethanol and shaken with a laboratory rotary shaker at 500 rpm for 2 h at 25°C. The supernatants were centrifuged at 10,000×*g* for 10 min at 4°C in a centrifuge (Eppendorf 5417R; Eppendorf, Hamburg, Germany). The mixture extract was hydrolyzed using 6 N HCl at 110°C for 24 h prior to chromatographic separation (23). The free amino acid analysis of the hydrolyzed sample was conducted using a Biochrom 30 series amino acid analyzer (Biochrom Ltd.) according to the method reported by Xu *et al*. (23) using a lithium high performance column (20 cmx0.45 cm, i.d.). The free amino acid was post-column derivatized with ninhydrin reagent and detected by absorbance at 570 nm. The total running time was 70 min with a flow rate of 0.4 mL/min using lithium citrate buffer and the free amino acid content was expressed in mg/g of fresh sample.

Determination of total phenolic content Total phenolic content in 80% methanol extract was determined using the Folin–Ciocalteu method reported in a previous research (24). The diluted extract with 80% methanol was shaken for 1 min and then 0.2 M Folin–Ciocalteu (1.0 mL) was added. After 3 min, 15% sodium carbonate solution (800 μL) was added and the entire solution was incubated for 30 min at room temperature. The absorbance of the solution was measured at 750 nm and all terminations were carried out in triplicate. The data were obtained by reporting the absorbance from the gallic acid standard curve (100, 250, 500, 750, and 1,000 mg/L) and the result was expressed as mg of gallic acid equivalents (μg GAE/g).

Antioxidant activities against DPPH radical The DPPH radical scavenging effect of 80% methanol extract of sample was determined by the method reported by Kim *et al*. (18). In more detail, the methanol extract (0.1 mL) or positive control (butylated hydroxytoluene (BHT)) of different concentrations was added with 0.49 mL methanol followed by 0.39 mL of 1 mM DPPH methanolic solution. The crude mixture was vortexed for 1 min and incubated for 30 min in darkness at room temperature. Decrease in absorbance was evaluated at 517 nm. This activity was measured as a percentage using the following formula:

$$\text{DPPH radical scavenging effect (\%)} = (1 - A_{samp}/A_{cont}) \times 100,$$

where A_{samp} is the absorbance of the sample and A_{cont} is the absorbance of the control.

Statistical analysis All measurements were made in triplicate. The results were subjected to variance analysis using Sigma plot 2001 (Systat Software Inc., Chicago, IL, USA). The lignin and free amino acid contents were expressed as a mean.

Results and Discussion

Comparison of phytochemicals in sesame seeds during germination Phytochemicals are widely distributed in fruits, vegetables, grains, and other food products (2,12) and are classified as phenolics, alkaloids, triterpenoids, and carotenoids (6,19,21). Furthermore, many researchers are currently identifying new pharmacological activities of phytochemicals with respect to their nutritional and functional values to enhance the potential uses (3,9,20). In addition, many studies have demonstrated that various processing methods such as germination and fermentation increase nutraceutical values and health-promoting phytochemicals in natural sources (2,3). Specifically, germination is considered as an effective and inexpensive technology to promote nutritional quality (5). For these reasons, we investigated and compared the phytochemical amounts in different colored sesame seeds (white and black) and their sprouted seeds upon germination. Using the correct extraction condition is an important first step in the isolation of phytochemicals (25). Although many factors contribute to the efficiency of extraction, the identity of the extraction solvent is key because solvents have the highest effects on phytochemical extraction from natural sources (25). Among the various solvents, methanol is found to be an appropriate and effective solvent for the maximum extraction of phenolic compounds (25). Moreover, the combination of methanol with water showed an excellent effect on lignan extraction in our previous study (18). Therefore, we quantified phytochemical contents from an 80% methanol extract of sesame seeds. The 18 phytochemical contents were examined and their chemical structures are shown in Fig. 1.

A representative HPLC chromatogram for the phytochemicals was generated within 30 min at a wavelength of 254 nm (Fig. 2A). The

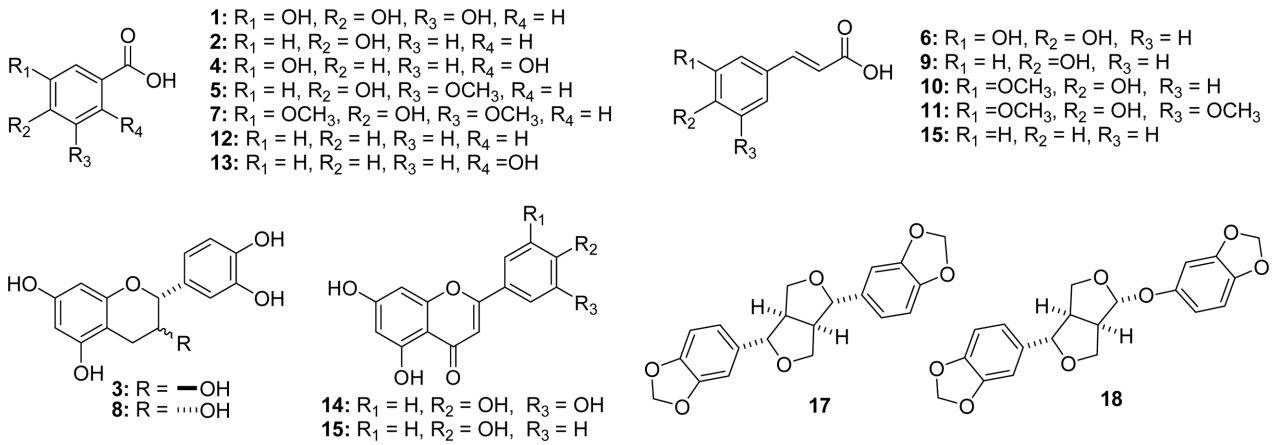

Fig. 1. Chemical structures of phytochemicals characterized from raw sesame seeds and their sprouted seeds.

phytochemical retention times are as follows: peak 1 (gallic acid, t_R=5.3 min), peak 2 (*p*-hydroxybenzoic acid, t_R=10.2 min), peak 3 (catechin, t_R=10.9 min), peak 4 (gentisic acid, t_R=11.6 min), peak 5 (vanillic acid, t_R=12.3 min), peak 6 (caffeic acid, t_R=12.8 min), peak 7 (syringic acid, t_R=13.7 min), peak 8 ((-)-epicatechin, t_R=14.3 min), peak 9 (*p*-coumaric acid, t_R=15.7 min), peak 10 (ferulic acid, t_R=16.9 min), peak 11 (sinapic acid, t_R=17.2 min), peak 12 (benzoic acid, t_R=17.7min), peak 13 (salicylic acid, t_R=19.2 min), peak 14 (luteolin, t_R=20.4 min), peak 15 (cinnamic acid, t_R=21.3 min), peak 16 (apigenin, t_R=22.1 min), peak 17 (sesamin, t_R=28.1 min), and peak 18 (sesamolin, t_R=29.3 min). Identification of the phytochemicals in samples was conducted by comparing their retention times to those of standards. Changes in phytochemical contents of sesame seeds through germination are presented in Table 1. The individual and total contents exhibited remarkable differences between colored sesame seeds and their sprouted seeds. In the case of white sesame (cv. Kopum), sesamin (**17**) and sesamolin (**18**) were observed as major compounds at 4.21 and 3.57 mg/g, respectively. Sesamin (**17**) was especially the predominant component, representing approximately 54% of the total content. Our result is similar to that of a previous study reporting that sesamin was found in large amounts (18). The remaining 16 components were not detected. For germination, the harvested white seeds were placed in a growth chamber for 7 days at 25±1°C with 80% humidity. The sprouted seeds exhibited considerable differences in comparison with raw sesame seeds (Fig. 2B). Interestingly, sesamin (**17**) and sesamolin (**18**) decreased more than two times to 1.72 and 1.57 mg/g, respectively, during germination. The most predominant composition was catechin (**3**) at 13.50 mg/g, representing approximately 57.1% of the total content (23.65 mg/g). (−)-Epicatechin (**8**) was the second major compound at 2.30 mg/g (9.7%), followed by sinapic acid (**11**) (2.13 mg/g, 9.0%), salicylic acid (**13**) (1.24 mg/g, 5.2%), and luteolin (**14**) (0.86 mg/g, 3.6%), whereas benzoic acid (**12**) exhibited the lowest content (0.33 mg/g, 1.4%). Other phytochemicals observed in raw seeds were not observed in sprouted seeds. These results suggest that the phytochemical profile of sesame seeds may be positively correlated with environmental factors, including processing methods (5,6). Previous studies have also revealed that thermal processing and heat or pressure treatment have influenced the phytochemical contents and their compositions because of their hydrophilic properties, transformations, degradations, and chemical reactions (26,27). Therefore, changes in phytochemicals of sesame seeds during germination observed in this study agreed well with earlier researches on the increase of metabolites in various crops (6). Phytochemical profiles of black sesame seeds also exhibited similar patterns as those of white sesame (Fig. 2C). The lignan content (3.01 mg/g) in raw black sesame seeds was detected to be two times lower than that in white seeds (7.78 mg/g) (Table 1). Although the number of sesame cultivars was small, our results were similar to those of earlier studies reporting that sesamin was found in large concentrations, whereas sesamolin was present in small amounts (18). During germination, the phytochemical contents displayed significant variations (Fig. 2C and Table 1). In more detail, this processing skill resulted in an increase in the total content (26.31 mg/g) compared with the raw black seeds at 3.79 mg/g. The major components identified were catechin (**3**) (19.09 mg/g), sinapic acid (**11**) (2.94 mg/g), and (-)-epicatechin (**8**) (2.34 mg/g), similar to those of white sesame (Table 1). Although benzoic acid (**12**) (ND→0.08 mg/g), salicylic acid (**13**) (ND→0.19 mg/g), and luteolin (**14**) (ND→ 0.34 mg/g) exhibited a slight increase, the lignan contents of sesamin (**17**) and sesamolin (**18**) were found to have considerably decreased from 2.43→1.36 and 0.58→0.45 mg/g, respectively (Table 1). According to the above evidences, lignan compounds may be considerably influenced by environmental stresses, including the germination process (6,26). Our study agreed well with earlier studies that the germination process was observed to result in significant changes in the phenolics owing to differences in endogenous enzyme conditions and variations in biochemical metabolisms (3,5). We report here for the first time that variations in phytochemicals such as phenolics and lignans occur in sesame seeds during germination.

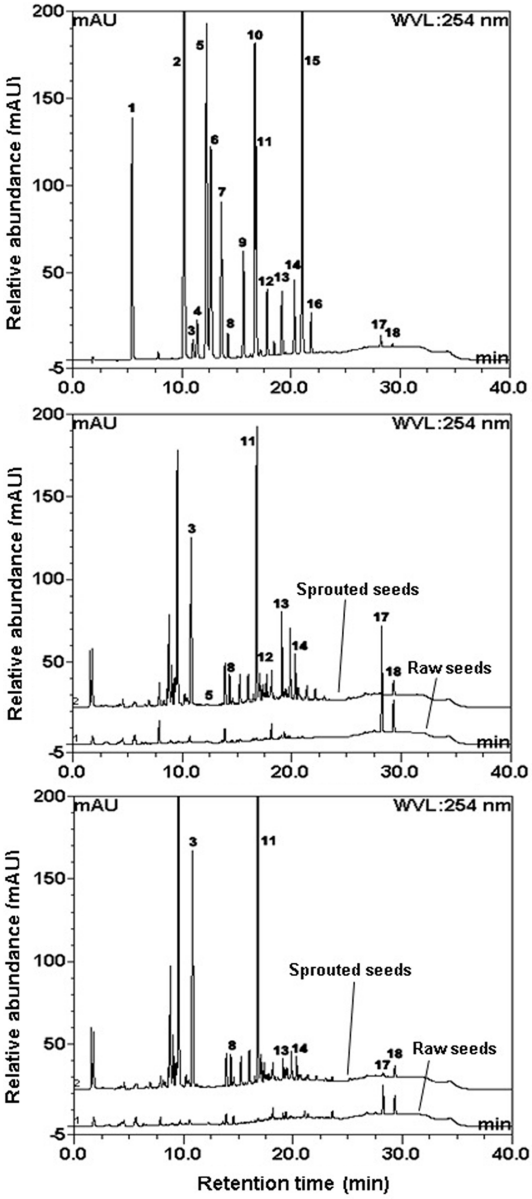

Fig. 2. Typical HPLC chromatograms of phytochemicals. (A) HPLC chromatogram of phytochemical standards, (B) HPLC chromatograms of 80% methanol extracts of raw white sesame seeds and their sprouted seeds, (C) HPLC chromatograms of 80% methanol extracts of raw black sesame seeds and their sprouted seeds.

Comparison of free amino acids in sesame seeds during germination Free amino acid is an essential parameter in investigating the qualities of foods and crops as well as their nutritional values (28). Although previous studies are available on free amino acid compositions (14,26), to our knowledge, there is no information about the variations in free amino acids in different color coated sesame seeds during germination.

The 21 free amino acids were quantified in both raw and sprouted sesame seeds using an amino acid analyzer and their profiles are presented in Table 2. The individual and total amino acid contents showed similar patterns in raw white and black sesame (Fig. 3A). Briefly, this constituent was observed to be slightly different in both seed colors with values of 7.04 mg/g (white sesame) and 6.55 mg/g (black sesame), respectively. However, the individual amino acid content varied depending on the seed colors and germination process. In raw white seeds, Asn had the most predominant composition with 1.69 mg/g, which is approximately 24% of the total amino acid contents, followed by Glu (0.58 mg/g), Leu (0.55 mg/g), Phe (0.45 mg/g), Ala (0.44 mg/g), Tyr (0.39 mg/g), Val (0.33 mg/g), and Lys (0.31 mg/g) (Table 2). Other amino acids displayed minor contents with a range of ND–0.30 mg/g. We found that free amino acid content significantly increased during germination (Fig. 3 and Table 2), i.e., total content increased by 4.5 times (7.04→31.69 mg/g) more than that of raw white seeds (Fig. 3A). Among the individual composition, Thr content increased significantly (12 times, 0.12→1.45 mg/g) and the remaining amino acids increased with high rates of 2–6 times upon germination. Especially, Asn, Arg, Leu, and Ala had predominant contents with 4.52, 4.11, 2.47, and 2.16 mg/g, representing approximately 14.3, 13.0, 7.8, and 6.8%, respectively (Table 2). Also, the sprouted seeds were observed to be especially rich in Trp and l-Mhis with their values changing from ND→0.43 and ND→2.51 mg/g, respectively, when compared with raw white sesame (Table 2).

In the case of black sesame seeds, Asn was observed to be the most predominant component at 1.26 mg/g, followed by Glu (0.59 mg/g), Leu (0.55 mg/g), Arg (0.50 mg/g), and Phe (0.46 mg/g), whereas the remaining components displayed minor amounts (<0.45 mg/g) (Table 2). During germination, the content of total amino acid increased significantly from 6.55→26.97 mg/g (Fig. 3A). The individual contents (except GABA: 0.21→0.21 and Ethamin: 0.23→0.39 mg/g) also increased by more than two times compared with those of raw black seeds (Fig. 3B). The most abundant composition was Arg accounting for 3.41 mg/g followed by Asn, Leu, and Trp present in large amounts at 3.17, 2.22, and 2.26 mg/g, respectively (Table 2). Furthermore, l-Mhis and Trp were not found in raw seeds but they were present in considerable amounts at 0.47 and 2.26 mg/g, respectively, in their sprouted seeds. In particular, Arg and Thr contents exhibited high variations of approximately 6.8 (0.50→3.41 mg/g) and 6.1 (0.19→1.15 mg/g) times (Table 2). Thus, the current study illustrated that the free amino acid content of sesame seeds increased during germination (Fig. 3B). Our results agreed well with those of previous studies reporting that free amino acids can be affected by numerous factors, including process techniques, environmental stresses, agronomic conditions, genetics, and cultivation skills (6,18, 19,21). This research is the first to evaluate free amino acid contents in raw and sprouted sesame seeds.

Comparison of total phenolic contents in sesame seeds during germination The amount of total phenolics in the extracts of raw sesame seeds and their sprouted seeds are shown in Fig. 4. Black sesame seeds exhibited higher content at 645.18±31.5 μg GAE/g than white sesame (503.10±27.1 μg GAE/g). This phenomenon may

Table 1. Changes in phytochemical contents of different colored sesame seeds and their sprouted seeds upon germination

Peak	Phytochemical	t_R (min)	Content (mg/g)[1)]			
			White sesame[2)]		Black sesame[3)]	
			Raw seeds	Sprouted seeds	Raw seeds	Sprouted seeds
1	Gallic acid	5.3	ND[4)]	ND	ND	ND
2	*p*-hydroxybenzoic acid	10.2	ND	ND	ND	ND
3	Catechin	10.9	ND	13.50	ND	19.09
4	Gentisic acid	11.6	ND	ND	ND	ND
5	Vanillic acid	12.3	ND	tr[5)]	ND	tr
6	Caffeic acid	12.8	ND	ND	ND	ND
7	Syringic acid	13.7	ND	ND	ND	ND
8	(-)-Epicatechin	14.3	ND	2.30	ND	2.34
9	*p*-Coumaric acid	15.7	ND	ND	ND	ND
10	Ferulic acid	16.9	ND	ND	ND	ND
11	Sinapic acid	17.2	ND	2.13	ND	2.94
12	Benzoic acid	17.7	ND	0.33	ND	0.08
13	Salicylic acid	19.2	ND	1.24	ND	0.19
14	Luteolin	20.4	ND	0.86	ND	0.34
15	Cinnamic acid	21.3	ND	ND	ND	ND
16	Apigenin	22.1	ND	ND	ND	ND
17	Sesamin	28.1	4.21	1.72	2.43	1.36
18	Sesamolin	29.3	3.57	1.57	0.58	0.45
	Total content		7.78	23.65	3.79	26.01

[1)]All values are presented as the mean of triplicate determinations, Content expressed as mg of each phytochemical equivalent per g of dry weight.
[2)]White sesame cultivar, Kopum
[3)]Black sesame cultivar, Mihuk
[4)]ND, not detected
[5)]tr, trace

be attributed to genetic properties and various metabolites in black sesame seeds (29,30). In sprouted sesame seeds, the total phenolic content showed a steep increase (p<0.001) compared with that in raw seeds. The sprouted white seeds exhibited markedly amounts, approximately 4 times higher, at 503.1±27.1→2085.0±56.7 μg GAE/g when compared with the raw seeds (Fig. 4). Black sesame was also observed to demonstrate a considerable increase of 3 times in raw seeds (645.8±31.5 μg GAE/g) compared with their sprouted seeds (2480.0±49.5 μg GAE/g) (Fig. 4). Our study suggests that the germination process may cause significant changes in the phenolic content and other biochemical characteristics. In addition, our results are similar to those of earlier researches reporting that bioactive compounds of soybean were found in large amounts depending on germination conditions (2,5,6). As a result, germination may considerably influence variations in phenolic contents through activation of endogenous enzymes and biochemical metabolism pathways of sesame seeds (22).

Comparison of DPPH radical scavenging effects in sesame seeds during germination Many researchers have focused on the antioxidant activities of crops, fruits, and vegetables (2-4). Specifically, the scavenging assay on DPPH radical is used in many fields because of its simple quality control and reproducibility (2,4,11,18). Thus, we investigated antioxidant properties against DPPH radicals using different extracts (10, 20, 40, 80, 100, 200, and 400 μg/mL) of raw sesame seeds and their sprouted seeds. The antioxidant abilities in various concentrations of samples were determined by comparing the percentage inhibition in forming DPPH radical using BHT (positive control). The DPPH radical scavenging capacities of raw and sprouted seeds as well as BHT increased with increasing concentration. Although 100% radical scavenging effect was observed at 400 μg/mL, the antioxidant abilities of the radical scavengers were tested at 200 μg/mL because of changes in the inhibition rates of the sample. BHT exhibited higher scavenging activities than raw sesame seeds and had fewer effects than the sprouted seeds. Furthermore, black sesame seeds demonstrated higher scavenging capacities than white seeds. Although the phytochemical content of white sesame (7.78 mg/g) was higher than those of black seeds (3.79 mg/g), other metabolites of black sesame may be responsible for the scavenging activities against the DPPH radical (Total phenolic content, black: 645.18±31.5 μg GAE/g, white: 503.10±27.1 μg GAE/g). Our results suggest that the different metabolites in sesame seeds with black coats may be correlated to the radical scavenging capacities as previously reported (18). The antioxidant effects of the 80% methanol extract in black seeds at different concentrations were as follows: 12% at 10 μg/mL, 18% at 20 μg/mL, 29% at 40 μg/mL, 45% at 80 μg/mL, 55% at 100 μg/mL, and 71% at 200 μg/mL. Moreover, the sprouted black seeds were observed to have higher scavenging effects than raw seeds (20% at 10 μg/mL, 33% at 20 μg/mL, 46% at 40 μg/mL, 64% at 80 μg/mL, 72% at 100 μg/mL, and 91% at 200 μg/

Table 2. Changes in free amino acid contents of different colored sesame seeds and their sprouted seeds upon germination

No.	Free amino acid	Content (mg/g)[1)]			
		White sesame[2)]		Black sesame[3)]	
		Raw seeds	Sprouted seeds	Raw seeds	Sprouted seeds
1	Asp	0.14	0.81	0.14	0.65
2	Thr	0.12	1.45	0.19	1.15
3	Ser	0.27	1.45	0.24	1.18
4	Asn	1.69	4.52	1.26	3.17
5	Glu	0.58	1.74	0.59	1.80
6	Gly	0.19	0.54	0.17	0.39
7	Ala	0.44	2.16	0.38	1.57
8	Val	0.33	1.83	0.31	1.46
9	Met	0.15	0.58	0.16	0.53
10	Ile	0.25	1.28	0.25	1.07
11	Leu	0.55	2.47	0.55	2.22
12	Tyr	0.39	1.20	0.30	1.19
13	Phe	0.45	1.39	0.46	1.40
14	GABA	0.14	0.22	0.21	0.21
15	Ethamin	0.18	0.41	0.23	0.39
16	Amm	0.21	0.49	0.21	0.42
17	Lys	0.31	1.16	0.28	1.27
18	l-Mhis	ND[4)]	0.43	ND	0.47
19	His	0.14	0.94	0.12	0.76
20	Trp	ND	2.51	ND	2.26
21	Arg	0.51	4.11	0.50	3.41
Total content		7.04	6.55	31.69	26.97

[1)]All values are presented as the mean of triplicate determinations, Content expressed as mg of each phytochemical equivalent per g of dry weight.
[2)]White sesame cultivar, Kopum
[3)]Black sesame cultivar, Mihuk
[4)]ND, not detected

mL). The sprouted black seeds also exhibited higher radical scavenging effects than those of sprouted white seeds. In particular, the extract of sprouted black sesame (200 μg/mL) showed slightly higher DPPH radical scavenging abilities than BTH. Therefore, germinated black sesame seeds may be an important source for nutraceutical and functional foods in preventing free-radical mediated diseases. On the basis of the above evidences, the scavenging capacities of DPPH radical occurred in the following order: sprouted black seeds>sprouted white seeds>raw black seeds>raw white seeds at a concentration of 200 μg/mL. Our results have confirmed that the formation and transition of many phenolic compounds through germination may be responsible for the scavenging activities against the DPPH radical (22).

In conclusion, this research has evaluated for the first time the changes in nutritional compositions (phytochemical and free amino acid) and antioxidant effects in white and black sesame seeds during germination. Phytochemicals and free amino acids in sprouted sesame were detected in significantly higher amounts compared with those detected in raw seeds. Particularly, catechin, Asn, and Arg were observed as the predominant components in sprouted seeds. These three amino acid components displayed remarkably high variations between raw and sprouted seeds with ND→13.50 mg/g (white seeds); ND→19.09 mg/g (black seeds) [catechin], 1.69→4.52 mg/g; 1.26→3.17 mg/g [Asn], and 0.51→4.11 mg/g; 0.50→3.41 mg/g [Arg], respectively. Total phenolic content increased by approximately four times from 503.1±27.1→2085.0±56.7 (white) μg GAE/g and 645.8±31.5→2480.1±49.5 μg GAE/g (black) in sprouted sesame. The antioxidant capacities against DPPH radical in sprouted seeds were also stronger than those of raw sesame. Our study reveals that germinated sesame seeds may be considered as a very

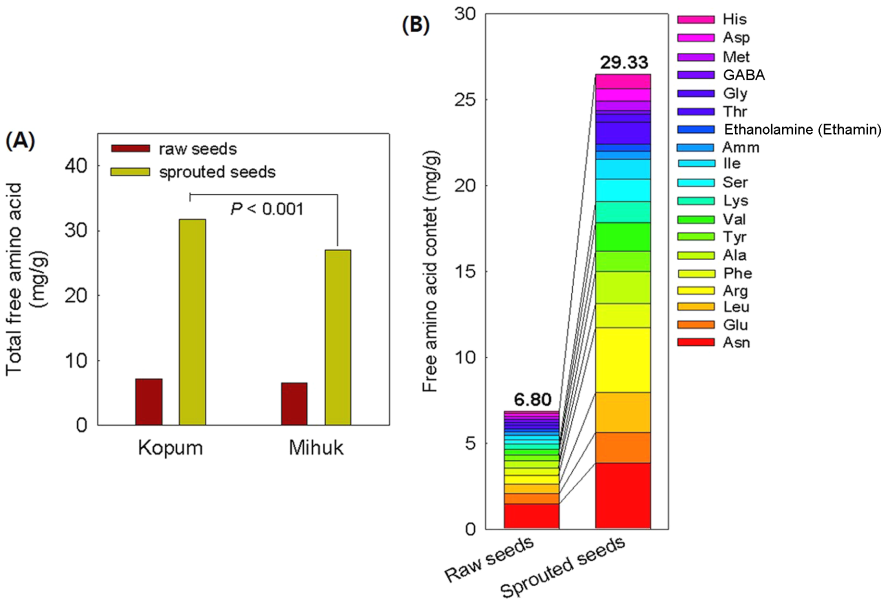

Fig. 3. Comparison of individual and total free amino acid contents in sesame seeds during germination. (A) Total free amino acid contents in raw white and black sesame seeds and their sprouted seeds, (B) Individual free amino acid content in raw and sprouted sesame seeds.

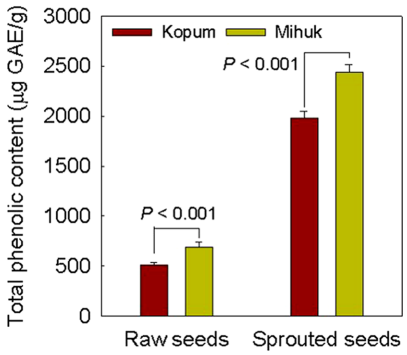

Fig. 4. Comparison of total phenolic contents in white and black sesame seeds during germination.

valuable natural source for functional foods and pharmaceutical uses because of their high phytochemical and free amino acid contents and high radical scavenging abilities. We believe that the germination process is an effective technology for improving nutritional qualities and biological effects of foods. For the development of potential health products and nutraceutical materials, future research is still needed to investigate the various metabolites and their beneficial biological effects in more detail.

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